TISSUE CULTURE OF *PINGUICULA*

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Butterworts (*Pinguicula* sp.) are usually propagated by seed, leaves or occasional sideshoots. Tissue culture is a relatively new method. With proper equipment and sterile laboratory techniques almost any plant can be tissue cultured.

The most difficult aspect of tissue culture is the elimination of bacterial and fungal contaminants. For most species of *Pinguicula* I have worked with*, it is necessary to take out the shoot meristem. This is the very center of the plant, where the new leaves emerge. When all other leaves and roots are cut away there is much less chance of contamination.

As a first step to obtaining clean tissue, the shoot is washed with soap and water. Next the shoot is trimmed again so that only unopened leaves remain at the tip. By now the shoot is less than one centimeter in height. It is placed in a solution of .5% sodium hypochlorite (10% chlorine bleach such as Chlorox). Adding a drop of liquid soap per twenty milliliters of solution helps the chemical sterilize the plant tissue. After shaking for fifteen to twenty minutes, the tip is removed in a sterile environment and rinsed twice in sterile water.

A sharply pointed scalpel and forceps are used to cut out the center of the shoot tip, one to two millimeters in size. This is the meristem, a white or colorless group of cells usually shaped like a somewhat rounded, microscopic pyramid. Placed on the right combination of nutrients and plant hormones,* protected from contaminating organisms, and supplied with optimum light and temperature, the tissue will thrive.

When the tissue grows to about 1 centimeter in height, it can be divided and placed on other nutrient media having different amounts of hormones or other chemicals. Sometimes a lot of tissue is

P. lutea, pumila, caerulea, ionantha, planifolia, primuliflora, gypsicola, lilicina.

* See Table 1.

Table 1. MEDIA INGREDIENTS FOR *PINGUICULA*

		milligrams/liter
$CaNO_3$	Calcium Nitrate	1000
NH_4NO_3	Ammonium Nitrate	300
KH_2PO_4	Potassium Phosphate	250
$MgSO_4$	Magnesium Sulfate	250
$MnSO_4$	Manganese Sulfate	10
Fe Chelate	Iron Chelate	20
Thiamine		10
Inositol		100
Sucrose		20,000
Agar		12,000

Plant hormones for shoot multiplication are Kinetin or 2iP in a range of 0.5 to 2.0 mg/liter of solution. Auxins for rooting were IBA or NAA in a range of 0.1 to 1.0 mg/liter.

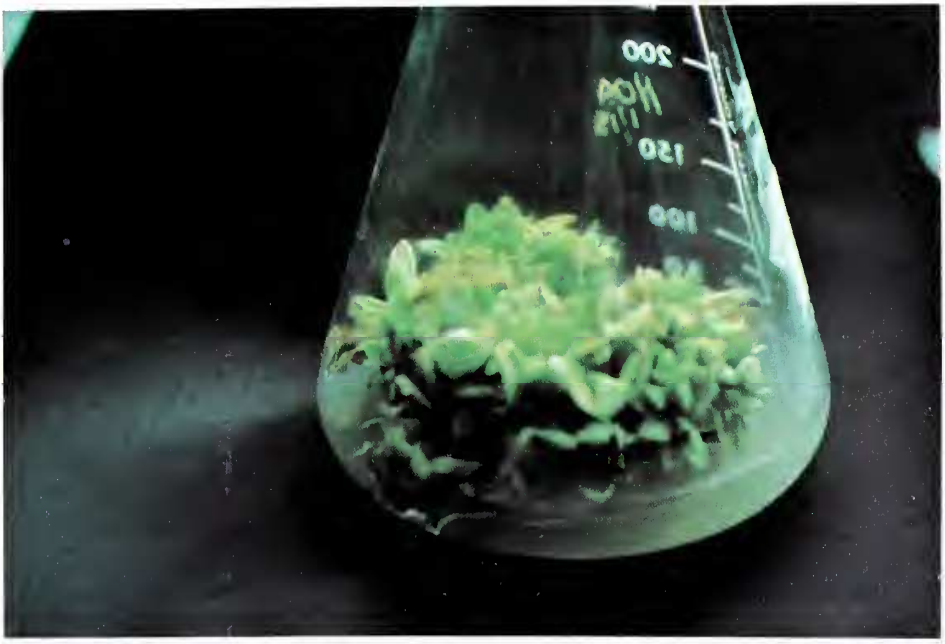
The media is brought to a boil while stirring constantly, dispensed into test tubes or other containers, and steam sterilized for fifteen minutes at fifteen pounds of pressure (120° C. or 250° F.).

Pinguicula pumila
Grown from seed.
Photo by Bill Carroll.

Pinguicula pumila
Grown from tissue culture.
Photo by Susan Sizemore.

Compare seed vs. clone

Cloned *Pinguicula lutea* and cloned *Dionaea muscipula* in the greenhouse. Cloned plants appear normal.
Photo by Bill Carroll.



Shoot multiplication of *Pinguicula lutea in vitro.*

Photo by Bill Carroll.

Pinguicula caerulea
From leaf tissue.

Pinguicula ionantha.
Comparison of two media.

Photos by Susan Sizemore.

needed to find the right combination for shoot multiplication. Once this is discovered, there is no end to the number of plants that can be produced. Later, some plants are moved to nutrient media with root-inducing hormones at different concentrations. In about a month, the plants can be transferred to soil.

Tissue cultured plants are transferred to soil much like tender seedlings. The nutrient media is first washed off to prevent disease organisms from starting. Roots are gently covered and the plants watered in with a ¼ strength solution of liquid fertilizer. The container is covered with clear or opaque plastic or glass to reduce light and hold in humidity. This cover is removed a little more each day over seven to ten days. The plants gradually adjust to their new environment.

Laboratories worldwide now produce tissue cultured ferns, orchids, African violets, as well as many other tropical plants. Some rare plants are becoming plentiful. Countries that restrict imports of soil grown plants can now receive plants *in vitro* in sterile conditions, making more varieties available to everyone.

FURTHER READING

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WHY THEY DO OR DO NOT GROW

by Don Schnell

How many times have those of us who grow CP noted one or both of the following events?

An article appears in CPN or some other publication going into great explicit detail about how a particular CP species can be grown—indeed, the author may insist that it *must* be grown the way he outlines or your plants will surely expire. You smile smugly and comment that you have been growing the same species a completely different way and the plants are perfectly healthy, or you come across one or more additional articles, these perhaps describing completely different ways of growing healthy specimens.

The second event is that you read a superbly detailed article about how to grow a CP to exquisite perfection. Everything seems to be there in print—water, pots, soil, fertilizer or no, light, etc.—Everything. So you set out, follow the direction to the letter, and your plants promptly go under.

This has happened to all of us, and woe be to the poor author beleaguered with smug or angry letters, respectively.

Are the authors right? Are they wrong? Well, they may be neither, or both. It comes down to a matter of perspective. There seem to be few absolutes among life forms, and many relative factors affecting them. And after that five cents worth of philosophy, let's see what might have gone wrong—or right.

In the first instance, where the plant does not seem to be as fastidious and